

P3. COMBINATION ANALYSIS OF ACTIVATOR PROTEIN-1 FAMILY MEMBERS, Sp1 AND AN ACTIVATOR PROTEIN-2 α -RELATED FACTOR BINDING TO DIFFERENT REGIONS OF THE UROKINASE RECEPTOR (u-PAR) GENE IN RESECTED COLORECTAL CANCERS

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Introduction: Studies on the transactivation of genes via promoter elements have mostly been done on cell lines rather than resected tissues. This, however, is essential to address an in vivo or clinical relevance. We have previously shown tumor-specific binding of Sp1 and an activator protein (AP)-2 α related factor to promoter region -152/-135 of the metastasis-related u-PAR gene in 60% of in vivo resected cancer tissues. Cell lines have implicated an additional role, and potential synergism, of an AP-1 region (-190/-171) in u-PAR regulation. This study was done to (a) analyze AP-1 binding to this region in resected tumor and normal tissues, and define subgroups in which it is tumor-specific, and (b) to analyze transcription factor binding patterns to both promoter motifs in resected tissues, supporting synergism, and draw first prognostic conclusions.

Methods: In 103 patients with colorectal cancer, electrophoretic mobility shift assay/supershift analysis for u-PAR promoter region -190/-171 was done in tumors and normal tissues. In 71 patients, region -152/-135 was also analyzed. U-PAR protein was measured by ELISA.

Results: Tumor-specific AP-1 binding to region -190/-171 of the u-PAR promoter was found in 40% of patients. Subgroup analysis showed tumor-specific binding for c-Fos in 58%, for c-Jun in 50%, for JunD in 39%, and for Fra-1 in 4% of cases. AP-1 binding correlated significantly with u-PAR protein amounts in both normal and tumor tissues ($p < 0.001$), in contrast to a tumor-specific correlation with u-PAR of the AP-2/Sp1 region. In analyses for both promoter regions, 62% of cancers showed simultaneous binding for AP-1, AP-2, and Sp1, 11% for AP-1 and AP-2, 16% for AP-2 and Sp1, 4% for AP-2 only, 3% for AP-1 only, and 0% for Sp1 only. The binding of AP-1, AP-2, and Sp1 correlated significantly with each other ($p < 0.001$), the combination of AP-1 and AP-2 showing the highest correlation with u-PAR ($p = 0.008$). Preliminary survival analysis indicated a trend for poorer prognosis for binding of all three transcription factors.

Conclusion: This is the first study differentiating transcription factor binding to two important u-PAR promoter regions in a large series of resected tumors and normal tissues. The AP-1 site seems to be a less tumor-specific regulator than the Sp1/AP-2 motif. Nevertheless, data corroborate the hypothesis of synergism between both elements in resected tumors.

doi:10.1016/j.ejcsup.2006.04.063

P4. TUMOR SUPPRESSOR Pdc4 INHIBITS INVASION AND REGULATES UROKINASE-RECEPTOR (u-PAR) GENE EXPRESSION VIA SP-TRANSCRIPTION FACTORS

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Background: Tumor-suppressor Pdc4 has never been investigated as to a potential role in invasion/metastasis. The urokinase receptor (u-PAR) promotes invasion and metastasis and is associated with a poor cancer-patient survival. The present study was conducted: (1) to implicate a role of Pdc4 in invasion and u-PAR-regulation, (2) to describe first mechanisms by which this is achieved.

Methods: Diverse gastrointestinal carcinoma cell lines were screened for Pdc4- and u-PAR expression and protein amounts by Northern-, Western blot analysis respectively RT-PCR. To determine a potential regulation of the u-PAR promoter by Pdc4, CAT- and luciferase reporter assays using diverse u-PAR wildtype and deletions mutants were undertaken and potential cis-element were screened for transcription factor binding by EMSA. Invasion and intravasation influenced by Pdc4 were tested by using different invasion assay approaches.

Results: In colon/gastric cancer cell lines, a reciprocal expression of u-PAR and Pdc4 was observed. RKO and HCT116 colon cancer cells made to express Pdc4 showed a reduction in u-PAR-mRNA and -protein, this being paralleled by an inhibition of invasion/intravasation. A CAT-reporter driven by the wildtype u-PAR-promoter was reduced in constitutive activity with increasing Pdc4-expression. Deletion of a region containing a putative Sp-1 binding site at -402/-350 inhibited u-PAR-promoter regulation by Pdc4, this being paralleled by a reduction of Sp1 binding to this region in pdc4-transfected cells. Pdc4-transfected cells showed an increase of Sp3 binding to u-PAR-promoter region -152/-135, and the deletion of this region reduced the ability of Pdc4 to suppress u-PAR-promoter activity.

Conclusion: These data suggest Pdc4 as a new negative regulator of invasion and the invasion-related gene u-PAR. Furthermore, it is the first study to implicate Pdc4-induced gene expression via Sp3 induction and Sp1 suppression.

doi:10.1016/j.ejcsup.2006.04.064

P5. EVIDENCE OF Pdc4 AS A NOVEL MARKER FOR TUMOR DIAGNOSIS AND PROGRESSION IN RESECTED COLORECTAL CARCINOMAS

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Background: Pdc4 (programmed cell death protein4) is a potential tumor suppressor, expression of which is downregulated in various human tumor types. Additionally, Pdc4 is able to inhibit the neoplastic transformation in the JB6 mouse model. Pdc4 has been shown to inhibit translation of diverse regulatory factors important for neoplastic transformation. Moreover, it was shown that AKT phosphorylates Pdc4, causing nuclear translocation and inactivation of the latter. This suggests that Pdc4 activity is dependent on its cellular localisation. Up to now, Pdc4 protein expression and cellular localisation has not been analysed in a large series of patients with colorectal cancer (CRC).

Methods: We investigated the expression pattern and localisation of Pdc4 tumor suppressor protein in resected tumor and corresponding normal tissue in a series of 41 CRC patients (32 R0-resected) who did not receive neoadjuvant treatment, by Western blotting (WB) and immunohistochemistry (IHC). A separate semiquantitative score for ICH staining of the cytoplasm and nuclei was established. Preliminary analysis of Pdc4 expression and localisation was correlated with patient's clinical tumor stage (UICC) and with recurrence-free survival.

Results: In WB high overall Pdc4 amounts were detected in normal tissue in comparison to the tumor samples where the signal was significantly decreased ($p = 0.025$, Wilcoxon). IHC analysis revealed strong nuclear presence of Pdc4 in the apical cryptal epithelium of normal tissue, as opposed to the complete loss of nuclear expression in tumor tissue ($p = 0.001$, Wilcoxon). In normal tissue, loss of Pdc4 nuclear expression/increase of cytoplasmic Pdc4-staining was significantly associated with advanced UICC stages ($p = 0.027$, χ^2). Preliminary Kaplan-Meier-analysis (median recurrence-free survival time: 38 months, range: 1-74 months) showed a trend for loss of Pdc4 expression in the nuclei of the normal tissue to be associated with poor recurrence-free survival ($p = 0.09$, Breslow log rank).

Conclusion: This is the first clinical study that demonstrates a potential relevance of Pdc4 expression and localisation in resected colorectal tumors and corresponding normal tissue, for tumor diagnosis and progression. Further analysis of colorectal adenomas will be performed to study the role of Pdc4 localisation as a potential clinical marker for carcinogenesis in CRC.

doi:10.1016/j.ejcsup.2006.04.065

P6. PROTEINASES ACTIVATED RECEPTORS: EXPRESSION AND QUANTIFICATION IN COLON AND PROSTATE HUMAN CANCER

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Background: Proteinases activated receptors (PARs) are important members of the G protein-coupled receptors family. The proteolytic cleavage of their extracellular domain by serine proteases such as trypsin or thrombin, generates an autoactivating tethered-ligand. Thus, the corresponding activated G proteins trigger a cascade of downstream events leading to diverse cellular outcomes such as calcium signalling, cell adhesion, cell migration and mitogenesis. The role of proteases in promoting invasion of cancer cells and tumorigenesis have prompted us to study the expression level and localisation of the four known members of the PARs family and explore their role in cell proliferation.

Methods: First, by using quantitative RT-PCR, we have quantified: (i) the expression of each PAR in colon and prostate cancer cell lines, (ii) the PARs expression in 40 patients samples (colon or prostate normal tissue and cancer). Second, we have studied PARs tissue localisation in colon and prostate, by using immunohistochemistry staining on TMA samples. Finally, we investigated the role of the four members of PAR family (PAR1-4) in cell proliferation by stimulating cell lines in different conditions (various thrombin concentrations).

Results: Our results show that the expression profile of the PARs is different for each PAR and depend mostly of the cell lines characterisation (origin and stage). However, we demonstrate a constant overexpression of two PARs in every tested cell line, that was also found in tumor samples.

Immunohistochemical analysis shows PAR1 endothelial expression which was stronger in malignant tissues (prostate and colon samples), and weaker (prostate) or absent (colon) in normal tissues.

Conclusion: These results confirm the importance of PARs in cancer, whose expression pattern is likely to influence cancer cell behaviour in tumors.

doi:10.1016/j.ejcsup.2006.04.066

P7. COMPLETE COMPILATION OF MATRIX METALLO-PROTEINASE EXPRESSION IN HUMAN MALIGNANT GLIOMAS

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Background: Glioblastomas are the most common malignant brain tumors in adults characterized by very aggressive local growth and invasiveness. Tumor invasion into surrounding brain tissue is facilitated by increased expression and activity of matrix metallo-proteinases (MMPs), which may be marker for tumor aggressiveness. However, for several of the 23 human MMPs there are no or only very limited literature data available concerning expression by glioblastomas. Therefore, we screened an extensive panel of 15 low-grade astrocytomas and 15 glioblastomas in order to fill the gaps in our knowledge about MMP expression by these tumors.

Methods: Expression of MMPs was analysed by semiquantitative RT-PCR and immunostaining. Total RNA was used as template for RT-PCR. Immunostaining was performed on cryosections.